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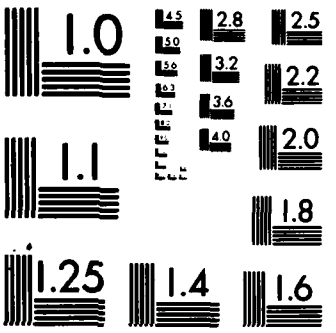

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RAPID IDENTIFICATION OF GROUP A STREPTOCOCCAL ANTIGEN DIRECTLY FROM THROAT SWABS: A STUDY USING TWO COMMERCIALLY PREPARED REAGENTS

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REPORT NO. 84-10

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RAPID IDENTIFICATION OF GROUP A STREPTOCOCCAL ANTIGEN DIRECTLY FROM THROAT SWABS: A STUDY USING TWO COMMERCIALLY PREPARED REAGENTS

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Report No. 84-10, supported by U.S. Army Medical Research and Development Command, Fort Detrick, Maryland, Department of the Army, under research Work Unit 3M162770A871.AB.306. The views presented in this paper are those off the author. No endorsement by the Department of the Army has been given or should be inferred.

Abstract

Presently, routine identification of pharyngitis caused by a streptococcal infection requires a 24-48 hour culture procedure. Direct identification of streptococci or streptococcal antigen from a throat swab would permit immediate identification. This study was to compare two commercial streptococcal latex reagents in demonstrating streptococcal antigen in swabs before culture. The latex products correctly identified 82% (Wellcome) and 91% (Difco) of the swabs containing streptococci as subsequently demonstrated by culture. The technology lends itself to the requirements of small laboratories or dispensaries and to field facilities such as those available to the Rapid Deployment Forces.

Rapid Identification of Group A Streptococcal Antigen Directly from Throat Swabs: A Study using Two Commercially Prepared Reagents

Introduction

Recent technology for rapid identification of group A streptococci has made it possible to identify group A streptocci while the patient is still in the "office". Moody et al (1) were the first to identify group A streptococci directly from throat secretions by a fluorescent antibody technique. El Kholy et al (2) were the first to demonstrate that group A streptococci could be identified from throat scrapings by extracting the scrapings with nitrous aid and identification using a precipitin test. Nitrous acid extraction has also been used to extract antigens from a single colony from a throat culture with a coagglutination test used for specific identification (3). The procedure for obtaining test material by throat scraping is uncomfortable for the patient, thus the procedure did not gain widespread use. Edwards et al (4) were able to identify group A streptococcal antigens from throat gargles. However the test population consisted of adults from whom gargles were easily obtained. Throat sampling from children for the presence of beta-hemolytic streptococci can best be accomplished by means of a cotton swab. Slifkin and Gil (5) were the first to show that streptococci could be identified directly from a swab. They used the nitrous acid extraction procedure to obtain antigen for testing. The reliability for detecting streptococcal antigen directly from a swab was further verified by Otero et al (6). These investigators used a "lytic extract" from Streptomyces griseus to digest or extract the group specific carbohydrate antigen from group A streptococci.

Our study was made to compare two commercially available reagents in their ability to detect group A streptococcal antigen directly from throat swabs.

MATERIAL AND METHODS

Sample population: Throat swabs were collected from 48 children attending the Pediatric Outpatient Clinic at Naval Hospital, San Diego. The collection of swabs for the "rapid testing" was highly selective in order to reduce the testing of large numbers of negative swabs and to conserve reagents. Patients ranged in age from 6 months to 9 years of age. All swabs were taken by one of the attending pediatricians at the Clinic.

Cultures for streptococci: Each swab (#MH 100, M & H Plastics, Inc., Redbluff, California, or Culture C.A.T.S. swabs from Precision Dynamics Corp., Burbank, California, both come with transport medium) was used to seed a sheep blood agar plate by rolling the swab over a small area at the edge of the plate. The inoculated area was then streaked by the use of a bacterial inoculation loop. The plates were incubated under reduced CO_2 at 37 C for 18-24 hours. Beta-hemolytic colonies suspected of being streptococci were further tested by measuring bacitracin sensitivity.

Testing for antigen direct from the swab: After inoculation of the sheep blood agar plate, each swab was placed in a 13 x 75mm disposable test tube. To this was added 0.4 ml of the extraction fluid (furnished with the Difco kits). The swabs were shaken in the extraction fluid by several thrusts against the palm of the hand and then allowed to "extract" for 45 minutes at 37 C. The swabs were pressed against the sides of the test tube to express fluid and "digested" bacteria (antigen) and then discarded. The extracted fluid was centrifuged in a bench centrifuge to pellet any debris that might interfere with test interpretation. The centrifugation was essential if the swab had been carried in any transport medium as small "flecks" of the transport medium in the test fluid would resemble aggregation of the test reagents and could result in an erroneous interpretation of the test.

TEST PROCEDURE

A drop (25ul) of the digested throat material was placed upon a dark glass plate (furnished with the kits). To this was added one drop (25ul) of the sensitized latex reagent (Lot #256-37-53 Difco, or #K6536 from Wellcome). Positive and negative controls were also run as standards (furnished with the kits). The digested material and latex were mixed with an applicator stick and the plate was rotated to and fro for 1-4 minutes before readings were recorded. A strong test would generally be positive within 1/2 to 1-1/2 minutes. (Care in handling the extracted material should be taken as it may contain viable organisms. All slides and stirring sticks should be properly disposed of.)

RESULTS

Of the 48 throat swabs collected, 22 grew beta-hemolytic streptococci with presumptive confirmation by bacitracin sensitivity. The latex product from Difco resulted in 23 positive tests and the latex product from Wellcome resulted in 21 positive tests (Table 1). Because we were selective in our sample population, a more accurate statistical analysis was not possible.

Table 1.

Comparison between cultures for beta-hemolytic streptococci and the latex test for streptococcal antigen directly from throat swabs.

		Difco Latex Reagent		Wellcome Latex Reagent			Difco Latex Reagent	
		+	-	+	-		+	-
Culture	+	20	2	18	4	Wellcome Latex Reagent	21	0
	-	3	23	3	23		2	25

DISCUSSION

It should be emphasized that the test procedure used in this study to detect streptococcal antigen was not the procedure recommended by the manufacturer in the use of the Wellcome product. The procedure we used was one recommended by Difco for a new product they were introducing to the market. Since their extraction procedure was to "release" antigen from the swab for subsequent detection by their latex reagent, it was of interest to us to determine if another latex reagent for detecting group A antigen would be similar in sensitivity and specificity. There is no immediate explanation for the latex reagents not detecting 100% of the positive cultures except that one of the cultures had but 4 single beta-hemolytic colonies present, suggesting a relationship in amount of infection and antigen detection. This relationship is seen in many infections. Less easily explained is the number of positive latex tests which were subsequently culture negative. We could not demonstrate a pattern of organisms that would cause false positives. One of the cultures was nearly a pure culture of Staphylococcus aureus but the latex test was negative. Another culture had a heavy growth of a Pseudomonas organism, the extract from this swab was negative by the latex tests. Since the latex tests described here do not rely on viable bacteria for identification, it seems possible that the patients from whom negative cultures/positive latex tests were obtained, may have been taking some antibiotic, which could have caused a negative culture.

These data firmly support the concept that group A streptococci can be rapidly identified directly from throat swabs. The use of commercial kits studied would provide the sensitivity, rapidity, and simplicity required by

either the smallest laboratory such as those in a dispensary or aboard ship, or a major central laboratory. The technology lends itself to the requirements of field laboratories of the Rapid Deployment Forces. Since our study was completed, a recent publication (7) supports our data in identification of streptococcus antigen directly from a throat swab by the latex test.

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 84-10	2. GOVT ACCESSION NO. AD-A140495	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) (U) Rapid Identification of Group A Streptococcal Antigen Directly from Throat Swabs: A Study Using Two Commercially Prepared Reagents		5. TYPE OF REPORT & PERIOD COVERED Final 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Earl A. Edwards		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Health Research Center P.O. Box 85122 San Diego, CA 92138-9174		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS U.S. Army 3M162770-A0871.AB.306
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Medical Research & Development Command NMCNCR, Bethesda, MD 20814		12. REPORT DATE March 1983 13. NUMBER OF PAGES 6
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) Commander, Naval Medical Command Department of the Navy Washington, DC 20372		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Approved for public release; distribution unlimited.

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Streptococcal disease
Rapid identification
Latex test
Pharangitis

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Direct identification of group A streptococcal antigens in extracts from 48 throat swabs, before culture, was done using a latex test. The latex method correctly identified 83% (Wellcome) and 91% (Difco) of the swabs containing streptococci as subsequently demonstrated by culture. The technology lends itself to the requirements of small laboratories or dispensaries and to field facilities such as those available to the Rapid Deployment Forces.

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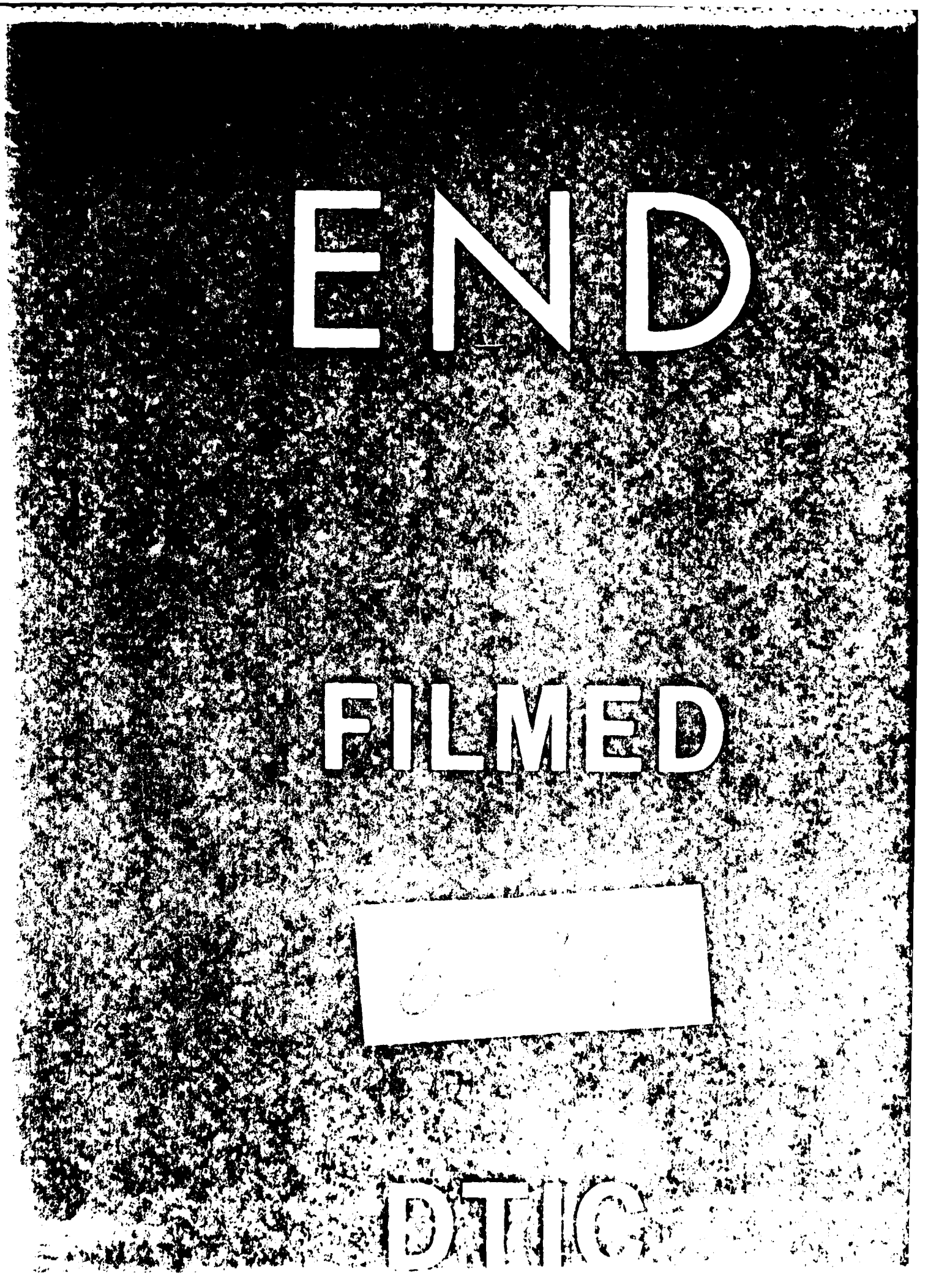
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